United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Role of fuel composition on smoke-induced cardiopulmonary toxicity neurobehavioural changes and genotoxicity in mice
LAPR Number:	18-08-002
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6/RTP/USEPA/US
Date Originated:	08/06/2015
LAPR Expiration Date:	08/31/2018
Agenda Date:	08/26/2015
Date Approved:	09/17/2015
Date Closed:	07/10/2018

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	09/17/2015	DMR	
	Exemption 6 Exemption 6 Exemption 6/RTP/USEPA/US by Exemption 6/RTP/USEPA/US	09/17/2015	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Role of fuel composition on smoke-induced cardiopulmonary toxicity neurobehavioural changes and genotoxicity in mice

Is this a continuing study with a previously approved LAPR?
No

2. Programatic Information
a. What Program does this LAPR support? Please provide the Research Program, Project, Task Number and Title.
ACE 183 (wildfire) PEP-1
b. What is the Quality Assurance Project Plan (QAPP) covering this project?
irp-nheerl/ephd/cib/2015-001-01

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	Exemption 6	EPHD	MD
	Lotus Notes Address	***Branch***	
	Exemption 6/RTP/USEPA/US	CIB	

4. Alternate Contact:

Alternate Contact	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	Exemption 6	EPHD	MD
	Lotus Notes Address	***Branch***	
	Exemption 6/RTP/USEPA/US		

SECTION A - Description of Project

1. Explain the study objective(s) in non-technical language such that it is understandable by non-scientific persons. Explain how the benefits from the knowledge gained from this research outweigh the costs to the animals used in this research. If this is a continuing study from a previous LAPR, briefly justify the

continuation. Please spell out all acronyms and abbreviations with their initial use.

Every year wildfires destroy thousands of homes, burn millions of acres, and threaten human life in the United States. Unfortunately, the scale and frequency of wildfires have been increasing over the past 30 years. Furthermore, since wood smoke is recognized by the World Health Organization (WHO) as a probable human lung carcinogen, health risks of short- and long-term exposures to wildfire smoke are becoming of growing concern not only for firefighters but also for the population impacted by smoke. The purpose of this project is to assess the relative cardiopulmonary and genetic toxicity and alterations in neurobehavior of biomass smoke emissions from four distinct fuel types (pine, peat, chaparral and mixed conifer) and further determine subchronic inhalation toxicity of the biomass smoke exposures, and provide a ranking of effect for the pulmonary, cardiac and mutagenicity assessments.

The rationale for this project is that biomass smoke emissions from different fuel types cause differential toxicity and could be used to identify toxic components within combustion emissions. These studies will monitor genetic, biochemical and cellular changes in the lung, heart and plasma of Big Blue mice after exposure to the biomass smoke. In addition pulmonary, cardiopulmonary, and neurobehavioral function testing will be assessed to determine whether these biomass emissions affect lung, heart, and brain mechanics.

Mice will be exposed to one concentration of each biomass smoke or air for up to 3 hours per day, 5 days a week for up to 13 consecutive weeks. Biomass smoke emissions will be generated from a glass tube traveling furnace system in Exemption 6 Exemption 6, diluted and transported into the animal facility Exemption 6 Exemption 6 through a sealed penetration in the fume hood and directed to inhalation exposure chambers. At 2 or 24 hours after exposure, mice will be tested for pulmonary, cardiopulmonary, and neurobehavioral functions before being euthanized and blood, lungs, heart and other tissues removed for analysis using immunologic, hematologic and pathologic techniques.

We have previously studied (and reported) acute inhalation toxicity responses to various types of woodsmoke, coal fly ash and different types of diesel and biodiesel blends as well as various types of ambient and source derived particulate matter. This LAPR extends the work of a previous LAPR (12-01-002: comparative toxicity of inhaled combustion emissions in mice) and more specifically focuses on subchronic inhalation toxicity responses to biomass smoke which are most relevant for risk assessment of wildland fire smoke.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

The use of whole animals is necessary to provide pulmonary, cardiopulmonary, and neurobehavioral response profiles after exposure to these smoke mixtures. The use of in vitro cell culture or tissue culture systems cannot replicate the complex interconnected nature of intact physiological systems.

b. Justify the species requested:

Big Blue mice have been recommended for the assessment of potential mutagenicity (capacity to cause mutations or genetic alterations) and genotoxicity (capacity to damage DNA) of exogenous compounds by the Organization for Economic Cooperation and Development (OECD) for many years as part of test guideline 488. Using this transgenic animal model, it is possible to quantitatively detect the mutations on chromosomal DNA in the cells of any organ. Big Blue is the only transgenic rodent mutation assay that can go deeper and test for mutagenicity at the DNA level, as well as determining the types of DNA damage caused. Furthermore, Big Blue mice can be used for standard pathophysiological measurements facilitating the assessment of multiple endpoints in each animals. Finally, there is a large toxicological database (acute and subchronic inhalation exposures) using the mouse as the subject species and this will provide the capability to compare toxicity data within a given species. Female mice will be used since they are more readily group housed.

3. How was it determined that this study is not unnecessary duplication?

While there have been some independent inhalation studies on biomass smoke (or woodsmoke), no studies have reported the subchronic and relative inhalation toxicity of biomass smoke emissions from different fuel types which represent wildland fires in the U.S. (based on a PubMed and Medline search dated 04/06/2015 containing the following key words: wildfire, biomass smoke, subchronic inhalation, mouse, fuel types, and combustion).

SECTION B - In Vivo Procedures

1. Briefly describe the experimental design. Include descriptions of the age, weight and sex of the animals. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

The objective of this study is to provide a comparative toxicity and genotoxicity of four distinct biomass combustion emissions. Pine, peat, chaparral, and mixed conifer will be tested to represent western and southeastern wildland fires in the U.S.

Simulated wildland fire burns will be conducted on a tube furnace system which consists of a quartz glass tube and controlled-speed traveling ring furnace. The tube furnace system is located in Exemption 6 Exemption 6, and biomass emission from the tube furnace will be delivered to either standard 0.5 cubic meter whole body inhalation chambers in which the mice are housed in individual wire compartments or to a nose-only inhalation chamber with animals being exposed whole body by use of a rat tube (with no restraint). Selection of the exposure chamber type will depend on on-going pilot testing of the combustion emissions to identify optimal flow and pressure conditions to achieve the desired aerosol mixture. In either scenario, target concentrations of particles, associated gases and climatic conditions will not be exceeded. Refer to section B8 for expected effects.

The same mass of fuels to be combusted will be held constant at 5-10 g in order to compare the potency of different biomasses (e.g., pine, peat, chaparral, and mixed conifer). Dilution air will then be introduced to produce the smoke atmosphere. Upper limits that will not be exceeded will be 400 mg per cubic meter of PM2.5 (particulate matter less than 2.5 micrometers in diameter) and 700 ppm of carbon monoxide. Dilution air will be manipulated and then after testing, held constant to maintain exposures below these parameters. Mice (12-14 weeks old, 20-25 g, female) will be exposed to clean air only, or the biomass smoke emission for up to 3 hours per day, 5 days a week for up to 13 consecutive weeks, and tested at 2 or 24 hours after 1, 4, or 13 weeks of exposure. Blood carboxyhemoglobin concentration in mice will be determined by submandibular bleeding in all cohorts of mice (Category C procedure). Pulmonary physiology and neurobehavioral testing (both Category C) will also be conducted in these cohorts of mice. To evaluate effects of biomass smoke exposure on lung physiology, mice will be placed into whole body plethysmograph chambers. Mice will be evaluated for neurobehavioral changes and motor activity in an open field.

After behavioral observation and pulmonary function testing mice will be euthanized and necropsied for tissue and fluid analysis.

To characterize effects of biomass smoke exposure on cardiac function, a smaller separate cohort of mice will be implanted with radiotelemeters and will be allowed at least ten days to recover before exposures commence (Category D procedure). Animals will then be euthanized at appropriate time points for subsequent tissue and fluid analysis

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

Power calculations have confirmed with historic data that the number of animals per treatment group is 8 mice to assure an adequate sample size required to obtain valid statistical results with pulmonary inflammation and associated effects.

For each fuel type, there will be a single concentration of the emission and an air control. Mice will be exposed 5 days per week for 1, 4, or 13 weeks, and assessments will be taken either 2 or 24 hours post-exposure.

One group of mice (8) designated for recovery will be assessed 7 days after the 13 week exposure.

We intend to assess three different exposure periods (1, 4, and 13 weeks) to compare acute and subchronic inhalation exposure outcomes.

Two time points are necessary since we have demonstrated that with this bioassay system some endpoints

occur relatively early (e.g., inflammatory cytokines at 2-6 hours) while other endpoints occur later (e.g., neutrophils at 24 hours).

Thus for each fuel type, there are 2 concentrations (air, smoke) x 3 exposure periods (1, 4, 13 weeks) x 2 time points (2, 24 hours) x 8 mice per time point = 96 (exposure) and 2 concentrations x 8 mice per concentration = 16 (recovery).

One group of mice (8) designated for radiotelemetry will be assessed periodically throughout the 13 week exposure, Thus for each fuel type, there are 2 concentrations x 8 telemetered mice per concentration = 16. Total 128 mice will be required for each fuel type. (See Table 1 in attachments)

There are four different fuel types. 4 x [96 (exposure) + 16 (recovery) + 16 (telemetry)] = 512 mice.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	*Adults*	*Offspring*
C) Minimal, transient, or no pain/distress:	448	
D) Potential pain/distress relieved by appropriate measures:	64	
E) Unrelieved pain/distress:	0	

4. Does this LAPR include any of the following:

☐ Restraint (>15 Minutes) ☒ Survival surgery

☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

a. Please provide a scientific justification. Describe how animals will be monitored, how health status will be tracked, and what records will be maintained.

Mice will be closely monitored during the postoperative period for abnormalities in electrocardiogram, heart rate, blood pressure, and body temperature regulation. And also mice will be continuously monitored telemetrically for signs of decrements in physiological function. Implantable telemetry is the only method available that enables the acquisition of reliable unconfounded electrocardiogram and blood pressure data in conscious unrestrained animals

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):

Biomass smoke inhalation: Mice will be exposed to biomass emissions for up to 3 hours per day either for one 4, or 13 weeks (5 exposures per week). Exposure concentrations will be in the range of 0 or 400 mg per cubic meter. Continuous gas and aerosol sampling for carbon monoxide, carbon dioxide, oxygen, total hydrocarbon, and particle mass concentration will be conducted from the chamber.

Following testing animals will be exposed by whole body in one of two inhalation chamber systems.

For whole body exposures mice will be housed in individual wire cages 2”x2”X4” and placed in a 0.5 cubic meter inhalation chamber operating at a minimum of 12 air changes per hour and operating at a slightly negative pressure.

The nose and mouth only inhalation chamber system is an alternative exposure apparatus that utilizes whole body exposures through the use of rat tubes. The port airflow rate will be maintained between 500 - 1000 mL/min which is well above the respiratory rate for any individual animal being exposed, and ensures a constant atmospheric concentration of pollutants. Mice are placed unrestrained in rat nose-only exposure tubes. The biomass smoke concentration entering the chamber will be precisely controlled and monitored, and the flow maintains a slightly positive static pressure in the chamber. To ensure no fugitive emissions and a safe environment for the exposure operators a secondary containment enclosure will be used.

In both cases the air temperature will be controlled in a range of 70-75 F, and humidity will be maintained between 30-70%.

b. Survival Blood Collections (method, volume, frequency):
Each mouse (20-25 g) will be bled for one time from the facial vein in order to measure carboxyhemoglobin immediately after 1, 4, and 13 weeks of exposure. Mice will be removed from the exposure chamber and gently restrained. The whorl (or cowlick) will be identified on the mouse cheek and the puncture performed using a 3 mm sterile lancet. Blood (up to 150 microliters) will be collected in a pipette and transferred to a container for assay. Pressure will then be applied with sterile gauze until bleeding has stopped.

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):
Pulmonary function tests: Airway responsiveness in unanesthetized, unrestrained mice. The day before exposure (pre), and 2 and 24 hours after exposure, assessments will be obtained on all mice using a whole body plethysmograph (Buxco) system to measure ventilatory parameters and the enhanced pause (Penh), an index of airflow limitation and a surrogate for bronchoconstriction. Additional measured parameters include tidal volume, frequency, inspiratory and expiratory flow rates, inspiratory and expiratory times, and minute ventilation. Accumulation of individual ventilator function data at these 3 time points will be collected for 30 min following a 5 min acclimation period for each mouse in its individual plethysmograph. Whole body plethysmography measurements will take place in A-building. The testing will not use any aerosol challenges (e.g. methacholine). Pulmonary function testing will be conducted by Exemption 6Exemption 6Exemption 6

Neurobehavioral function tests: Mice will be evaluated for neurobehavioral changes during exposures (clinical observations), immediately after removal from the whole body chamber (motor activity), and at around 2 and 24 hours after exposure ends. During smoke exposures, mice will be individually observed through the exposure chamber window and several specific observations will be noted or scored: level of activity, posture, respiration, exophthalmos (abnormal protrusion of the eyeball), coordination, cyanosis, facial swelling, convulsive or tremorigenic movements. Immediately after exposure, mice will be individually placed in plastic shoebox cages (with a small amount of Beta-Chip bedding) and the cages will be surrounded by a bank of photobeams that record locomotor and rearing activity. The cages will be placed on carts in the animal housing room, and as soon as mice are placed in them the room door will be shut. Locomotor testing is performed over a 60 min period in a closed room with no personnel present. At the end of the 60 min, personnel will enter the room and observe the animals for clinical symptoms as described above. No deleterious effects of these non-surgical procedures are expected. In the event that animals are seen to be suffering ill health they will be removed from the study and euthanized as per advice of the staff veterinarian. Mice will then be returned to their home cages. Neurobehavioral tests will take place in A-building, and will be conducted by Exemption 6 or other trained personnel.

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:
Inhalation exposures: whole body exposures will be conducted in stainless steel wire inhalation cage compartments (1.4"x1.5" x4") for up to three hours per day. Using the nose-only exposure chamber system, mice will be placed in rat tubes where they are free to turn around unimpeded. Duration of exposure in either case is up to three hours per day, 5 days per week for up to 13 weeks.

Pulmonary function tests: In this system the animal has complete freedom of movement and rests in a small clear plastic chamber (3.5" diam. x 2.5" ht.). Duration of testing is up to one hour one day per week for up to 13 weeks.

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):
n/a

f. Describe how animals will be identified and monitored. Include description of identification procedures. (For example, if transponders are used, how are the animals prepared?) Include frequency of observations and by whom:
All animals will be identified by implantation of subcutaneous microchips. Mice can be monitored visually through the clear Plexiglas window either inhalation chamber system and can be rapidly removed if needed. Laboratory staff will check the mice at the beginning of exposure and at 30-min intervals during the exposures. Animals will also be examined 1, 4 and 24 hours post exposure to ensure complete recovery.

Animals will be monitored each day during and after exposure and any animals displaying signs of illness (huddling, isolation with ruffled exterior, shivering, development of hindered movement, etc) will be assessed in consultation with on-site veterinarian. Animals will be monitored throughout exposures for any signs of ill health by inhalation personnel. During and after pulmonary assessment in the whole body plethysmograph, animals will

be monitored by staff to ensure they fully recover before being returned to animal housing. Routine monitoring will be performed daily by Exemption 6Exemption 6 or other trained personnel. No deleterious effects of these non-surgical procedures are expected. In the event that animals are seen to be suffering ill health they will be removed from the study and euthanized as per advice of the staff veterinarian.

All animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. The personnel responsible for monitoring include Exemption 6Exemption 6 . Animals will be weighed every 3 days and tracked for sudden weight loss (>10%). Representative animals from each treatment group will be monitored telemetrically for adverse effects on physiological function. If signs of distress or other deleterious effects are observed, animals will be isolated in a clean control atmosphere and observed for recovery trends. The attending veterinarian will be consulted when appropriate to determine the appropriate course of action. The personnel listed above will be responsible for monitoring animals during holidays and weekends.

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):
n/a

b. Blood Collection (Provide a description of the procedure including method, volume, and frequency if appropriate. Indicate if the procedure is survival or terminal. Include preparatory methods, descriptions of incisions, etc.):
n/a

c. Testing methods:
n/a

d. Restrictions placed on the animals' basic needs (e.g., food and/or water restriction, light cycles, temperature). Provide details regarding the length of restriction. Describe the method(s) for assessing the health and well-being of the animals during restriction. (Amount of food or fluid earned during testing and amount freely given must be recorded and assessed to assure proper nutrition.):
n/a

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):
n/a

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:
n/a

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:
n/a

7. Surgical Category D and E procedures. Indicate if the surgery is survival or terminal. Describe each surgical procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:
Surgical Implantation of electrocardiogram (ECG) biopotential (subcutaneous) radiotransmitters: A cohort of mice will undergo surgical implantation of sterile radiotransmitters (model: TA11ETA-F10, Data Sciences International) for the study of cardiac and thermoregulatory function. Before the implantation, the transmitter will be rinsed in sterile water, placed in the detergent (Terg-A-Zyme, 10 g of detergent powder in 1 L of water), and soaked for at least 4 hours. The transmitter will be then thoroughly rinsed in sterile water. Animals (~20-25 g) will be anesthetized (isoflurane) and implanted with sterile radiotelemetry transmitters; local anesthetic (lidocaine/bupivicaine – 1 mg/kg/1 mg/kg - 0.1 ml) will also be used at the incision site. Surgery will begin only after an appropriate anesthetic plane is reached (unresponsive to toe pinch). Briefly, the area where incisions will occur will be shaved using electrical clippers (Oster, Detachable Blade Animal Clipper), scrubbed with disinfectant solution (Betadine Solution), followed by cleansing with alcohol (PDI Alcohol Prep Pads) for a total of three times.

The transmitter portion of the device is ideally positioned subcutaneously along the dorsal flank. The animal will be placed in sternal recumbency on the surgery table. Using a small scalpel blade, a 1-1.5 cm midline

incision will be made through the skin on the dorsal back, cranial to the hind limbs. On the left side of the incision, a subcutaneous pocket will be made along the dorsal flank by blunt dissection. The pocket will be made a sufficient size to allow the transmitter to fit without it being too small, which can cause necrosis. The transmitter will be placed into the pocket with the ECG leads oriented cranially. Tissue hydration will be maintained throughout the procedure by injecting (slowly) 1.5 ml of warm sterile saline into the pocket. The electrode leads will be tunneled subcutaneously from the dorsal region to the ventral surface and secured with sutures (Ethicon; sterile 4-0 braided silk) in a modified lead II configuration (picture attached). The subcutaneous tissue will be closed with absorbable suture (Ethicon; sterile 4-0 Vicryl) and the skin with surgical staples; the incision will be moved laterally during the stapling process to avoid stapling over the spine. Aseptic technique will be observed for the surgeries, including the use of surgical drapes, sterilized instruments, gloves, and suture material. Masks will be worn. Body temperature will be supported during and after surgery using a recirculating warm water bath and warm water bottle, respectively. Analgesic (buprenorphine, 0.05 mg/kg × 2/day × 2 days) will be administered beginning immediately before surgery and postsurgically. Mice will be allowed at least 10 days to recover before exposures/treatments begin. While no deaths are anticipated due to these procedures, mice will be closely monitored during the postoperative period for abnormalities in electrocardiogram, heart rate, blood pressure, and body temperature regulation, and any animals judged to be in severe distress, will be euthanized. Surgery and monitoring will be performed by Exemption 6.

A surgical report will be included for groups of all animals undergoing surgery. This report will include details about quantity of anesthetic/analgesic administered, anesthetic depth, and body temperature and heart rate (HR) (taken from implanted telemeter). Ranges of body temperature and HR will be recorded; this will detail animal recovery until all animals are ambulatory and the time needed to get to this stage. All surgical instruments will be sterilized by autoclave at the end of the surgical day; instruments will be sterilized using a hot-bead germinator-sterilizer between animals for up to 5 animals (i.e., after the fifth animal, a new sterile pack of instruments will be started for the next animal). Cloth drapes will be used in the event that movement of drapes are necessary to facilitate surgery and will be sterile, and new per each animal.

Procedure for reattachment of telemeter leads: In the event that sutured leads come undone, they will be reattached with suture under isoflurane anesthesia (following assurance of anesthetic plane with toe pinch); buprenorpine will be administered for analgesia. If the telemeter leads are exposed, or the incision dehisces, a health report will be requested from animal care staff. The veterinarian will be consulted regarding post-operative complications, suture and antibiotic choice. Monofilament suture will be used if leads need to be re-sutured. There is usually no case in which leads are re-tunneled. Aseptic technique will be observed for this procedure, including the use of sterilized instruments, gloves, and suture material; body heat will be maintained with a recirculating warm water blanket. Masks will be worn.
*If animals require post-implantation surgeries to reattach leads or seal opened incisions, they will be provided with subcutaneous fluid (saline) support.

b. Anesthetic regimen (Drugs, dosages, volume, route of administration and delivery schedule). The use of paralytic or neuromuscular blocking agents w/o anesthesia is prohibited:

Isoflurane (3% per unit volume air): Inhaled surgical anesthetic for re-attachment of leads in the event they come undone. Inhaled isoflurane will also be used for telemetry surgeries.

Lidocaine/Bupivicaine: Local anesthetic (1% lidocaine/0.125% bupivacaine)

c. Postoperative care (thermal support, special feeding, responsible personnel, removal of sutures/staples, frequency and duration of monitoring including weekend and holiday care):

Surgical Implantation of Radiotelemetry Transmitters: Immediate postoperative care will be provided in Exemption 6 Exemption 6 Exemption 6. Analgesic will be administered before commencement of surgery (buprenorphine, 0.05 mg/kg) and postsurgically (buprenorphine e, 0.05 mg/kg × 2/day × 2 days). Postoperative antibiotic wound dressings will be applied following the surgery. Staples will be removed approximately ten days postsurgery. The personnel responsible for this care include: Exemption 6. Records of surgical and post-surgical care will be maintained by Exemption 6 in room A463-A. Following surgery, animals will be housed in Room Exemption 6 Exemption 6 Exemption 6. Animals will be monitored visually (obvious distress, gait, breathing, appetite, etc.) at least twice daily. Animals will be weighed on a regular basis and tracked for sudden weight loss (>10%). Animals will be continuously monitored telemetrically for signs of decrements in physiological function.

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):
Buprenorphine: Post-operative analgesic - 0.05 mg/kg administered once every 12 hours beginning immediately before surgery for a total of 2 doses and administered once immediately before re-attaching leads. Sustained release buprenorphine may also be used as an alternative.

e. Will any animal be subject to more than one surgical procedure over the course of its lifetime, either here at NHEERL or elsewhere?
○ Yes ● No

f. Identify any surgical procedures performed at other institutions or by vendors:
n/a

8. Humane interventions (for treatments/procedures in all categories).

a. What resultant effects, if any, do the investigators expect to see following procedures or treatment? Please include transitory as well as permanent effects. Examples might include lethargy, ataxia, salivation or tremors. Indicate the expected duration of these effects.
Biomass smoke is expected to cause acute airways irritation, lung inflammation, and genotoxic effects in tissue. Acute effects are expected to recover after the exposure although it is not known whether repeated exposures will result in cumulative injury. However, during smoke exposures, mice will be individually observed through the exposure chamber window and several specific observations will be noted or scored: level of activity, posture, respiration, exophthalmos (abnormal protrusion of the eyeball), coordination, cyanosis, facial swelling, convulsive or tremorigenic movements. Emergency contact information of the PI and Co PIs and tech staff and onsite Veterinarian will be displayed on the door entrance to Exemption 6 Exemption 6.

b. State the criteria for determining temporary or permanent removal of animals from the study. Describe actions to be taken in the event of deleterious effects from procedures or chemical exposures. Describe actions to be taken in the event of clinical health problems not caused by procedures or exposures.
Any animals displaying signs of illness (huddling, isolation with ruffled fur, shivering, development of hindered movement, greater than 10% weight loss etc) will be euthanized as per advice of the staff veterinarian.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.
Implantable telemetry is the only method available that enables the acquisition of reliable unconfounded electrocardiogram and blood pressure data in conscious unrestrained animals (based on a PubMed and Medline search dated 04/06/2015 containing the following key words: electrocardiogram, conscious, freely moving, blood pressure, mice, heart rate).

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	512
b. Animals to be transferred from another LAPR:	0
LAPR Number that is the source of this transfer:	
c. Animals to be transferred from another source:	0
d. Offspring produced onsite (used for data collection and/or weaned):	0
e. TOTAL NUMBER of animals for duration of the LAPR	512

2. Species (limited to one per LAPR): Mouse/Mice

3. Strain: Big Blue C57BL/6 Transgenic Mouse/Mice

Describe special requirements for animals with altered physiological responses (e.g., genetically altered, aged)

none

4. Sources of animals:

Taconic Biosciences

5. Provide room numbers where various procedures will be performed on animals:

Exemption 6: Housing animals, Whole-body plethysmography, and Telemetric monitoring of animals

Exemption 6: Intraperitoneal injections of animals in hood area, and Isoflurane anesthesia

Exemption 6: Neurobehavioral and pulmonary function tests

Exemption 6 Whole body biomass smoke exposures

Exemption 6 Anesthesia and telemetry surgeries

Exemption 6 Euthanasia, Necropsies, and Transmitter removal

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.

n/a *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)

n/a

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.

n/a

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

Implantation of identifier microchips

10. Housing and Enrichment.

The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.

For up to 3 hours per day exposed animals will be held in the inhalation chambers. The remaining time animals will be group-housed (4 per cage) in solid bottomed cages with beta chip bedding or other approved bedding. All mice will have access to nestlets for enrichment purposes. However animals undergoing telemetry surgery need to be housed singly so they do not open surgical incisions or remove ECG leads.

We will consider alternate housing options as well (e.g., one telemetered mouse with one untelemetered – with nestlets and 2 igloos) to improve animal well-being and outcomes.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.

Biomass combustion exposures will be up to 400 mg per cubic meter for total particulate (inhalation, up to 3 hours). There is no LC50 for biomass smoke inhalation exposures although studies have been reported with minor adverse effects for cigarette smoke at 800 mg per cubic meter. The LC50 for carbon monoxide in mice is 2444 ppm/4 hours. Carbon monoxide concentrations will be maintained below 700 ppm to avoid acute toxicity during exposures and no mortality is expected. Time of flight carbon monoxide will be used to monitor for and prevent acute toxicity.

Inhalation exposures will be conducted in inhalation chamber either operated under negative pressure or in a secondary enclosure to maintain a negative pressure for the chamber. For the inhalation system, a 5 minute flush period after exposure will be observed and inhalation chambers will not be opened until particle concentrations which are monitored continuously return to ambient levels. Gloves, lab coat and mask will be worn by personnel during all handling, transportation and experimental procedures including post-exposure cleanup.

Sodium pentobarbital (pharmaceutical grade): LD50 (oral) in mice is 239 mg/kg.

Buprenophine (pharmaceutical grade): LD50 (oral) in mice is 800 mg/kg

Heparin (pharmaceutical grade): LD50 (i.v.) in mice is 2800 mg/kg (100 units = 0.2 mg)

Isoflurane (pharmaceutical grade): LC50 (inhalation) in mice is 16,800 ppm. Isoflurane is considered a 'potentially hazardous substance' but does not require an HSRP. Isoflurane will be used in the chemical safety hood in Exemption 6, and standard PPE (safety glasses, gloves, lab coat) will be worn by all personnel at all times while being used.

Lidocaine (pharmaceutical grade): LD50 (oral) in mice is 292 mg/kg

Bupivicaine (pharmaceutical grade): LD50 (oral) in mice is 5866 mg/kg

2. Describe compounds to be administered to animals.

a. Are all substances pharmaceutical grade? If not, provide a scientific justification for the use of non pharmaceutical grade compounds.
Yes, except biomass smoke is an environmental agent and is thus not available in pharmaceutical grade.

b. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in the LAPR. Provide information to assure that such material is pathogen free. Indicate what safety precautions are necessary for handling the material.
n/a

c. Provide a statement regarding any safety precautions necessary for handling any of these materials.
Normal personal protective equipment (PPE) precautions will be observed throughout (gloves mask, labcoat, safety glasses). Inhalation exposures will be conducted under negative pressure to ensure safety of personnel

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	Study design, technical assistance and interpretation and reporting	has completed all NHEERL-required training, has over 20 years of experience conducting animal inhalation toxicology
Exemption 6	Associate Principal Investigator	Inhalation exposure and pulmonary toxicity assessment	has completed all NHEERL-required training, has over 6 years of experience conducting animal inhalation toxicology
Exemption 6	Associate Principal Investigator	Pulmonary toxicity assessment and veterinary input	has completed all NHEERL-required training, has over 20 years of experience in physiological testing
Exemption 6	Associate Principal Investigator	Cardiac toxicity assessment and radiotelemtery surgery	has completed all NHEERL-required training, has over 13 years of experience in physiological testing
Exemption 6	Associate Principal Investigator	Pulmonaryfunction assessment	has completed all NHEERL-required training, has over 20 years of experience in physiological testing
Exemption 6	Associate Principal Investigator	Genotoxicity assessment	has completed all NHEERL-required training, has over 20 years of experience in genotoxicity testing
Exemption 6	Associate Principal Investigator	Neurobehavioral function assessment	has passed all EPA animal and safety training, has over 30 years of experience in neurobehavioural testing
Exemption 6	Technical Staff	Neurobehavioral function assessment	has passed all EPA animal and safety training, has over 30 years of experience in neurobehavioural testing
Exemption 6	Technical Staff	Toxicity assessment, bleeding and necropsy	has completed all NHEERL-required training, has over 20 years of experience in toxicity testing
Exemption 6	Technical Staff	Inhalation exposures and animal husbandry	has completed all NHEERL-required training, has over 20 years of experience in inhalation testing
Exemption 6	Technical Staff	nhalation exposures and animal husbandry	has completed all NHEERL-required training, has over 20 years of experience in inhalation testing
Exemption 6	Technical Staff	Toxicity assessment,	has completed all NHEERL-required training,

		bleeding and necropsy	has over 20 years of experience in inhalation testing
Exemption 6	Technical Staff	Pulmonary function testing	has completed all NHEERL-required training, has over 20 years of experience in inhalation testing
Exemption 6	Associate Principal nvestigator	study design and execution	has completed all NHEERL-required training, has over 7 years of experience in animal toxicity and physiological testing
RTP-NHEERL	Tech Support	Category C Procedures	All NHEERL required training is complete.

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year 0

2. Breeding protocols and recordkeeping 00

3. Methods for monitoring genetic stability 0

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

2 and 24 hours following 1, 4, and 13 week exposures and 7 days after the final exposure (13 weeks). When mice are euthanized, blood will be also collected by cardiac puncture using a 1-mL syringe containing 0.1 mL (100 units) heparin to prevent clotting.

2. Describe the euthanasia techniques:

Method(s): Euthanasia plus exsanguination

Agent(s): Sodium pentobarbital (diluted euthasol)

Dose (mg/kg): Overdose of pentobarbital (150-250 mg/kg) followed by transection of abdominal aorta and vital organs

Volume: 0.025 mL/mouse for a 25 g mouse (200 mg/mL solution)

Route: : Intraperitoneal

Source(s) of information used to select the above agents/methods:

_ 2013 AVMA Guidelines on Euthanasia.

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

n/a

4. Describe how death is to be confirmed.

Vital organ section

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.
Euthanized as above
The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?
● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
4. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
5. The IACUC and Attending Veterinarian will be promptly notified of any unexpected study results that impact the animals' well-being, including morbidity, mortality and any occurrences of clinical symptoms which may cause pain or indicate distress.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. I certify that I am familiar with and will comply with all pertinent institutional, state and federal rules and policies.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 Exemption 6	09/11/2015

Submitted: 08/06/2015 ***Resubmitted:*** 09/11/2015

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	*Approval Date*	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	08/06/2015 by Exemption 6 Exemption 6 RTP/USEPA/US	Exemption 6 *Lotus Notes Address* Exemption 6 Exemption 6 /RTP/USEPA/US	*Branch*	MD *Submitted to Branch Chief for Approval* 08/06/2015 11:26 AM

ATTACHMENTS


ECG_lead II configuration.jpg


Table 1_LAPR_18-08-002.docx


18-08-002 PI Resp.pdf

Actions

First Update notification sent: 06/29/2016
Second Update notification sent:
First 2nd Annual notification sent: 07/03/2017
Second 2nd Annual notification sent:

1st Expiration notification sent: 06/28/2018
2nd Expiration notification sent:

History Log: